**LAB THREE: EFFECT OF TEMPERATURE ON NEURONS**

**OVERVIEW**

**In this lab you will:**

1. learn how changes in temperature affect neural spike firing
2. observe the temperature limits at which neurons cease firing

**OBJECTIVES**

**Before doing this lab you should study:**

* how action potentials are generated
* how sodium and potassium channels operate
* how temperature affects chemical reactions

**After doing this lab you should be able to:**

* explain the way that neurons produce action potentials
* describe why low temperatures would prevent a neuron from producing action potentials
* design an experiment to test for the optimal temperature range for neural firing

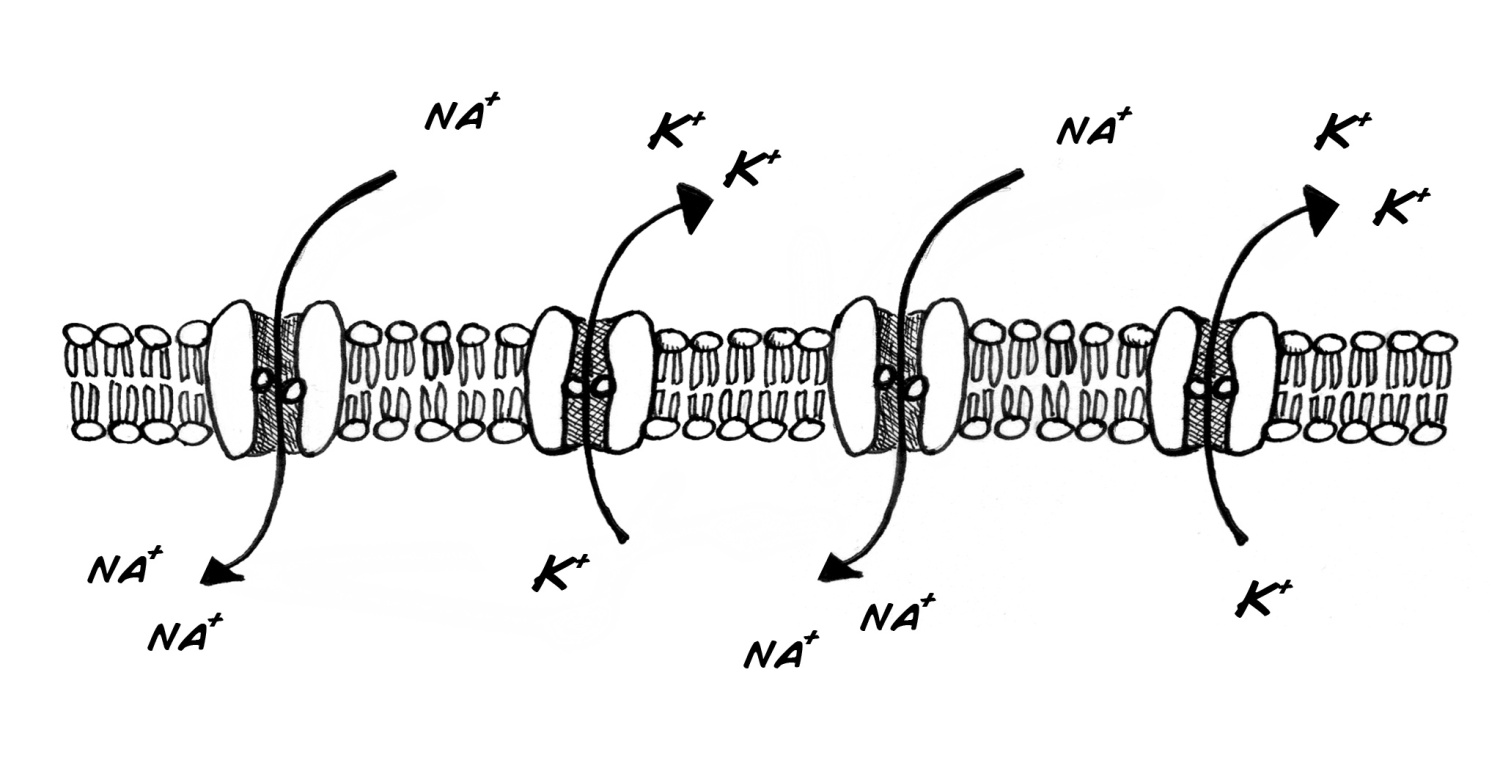
**EQUIPMENT**

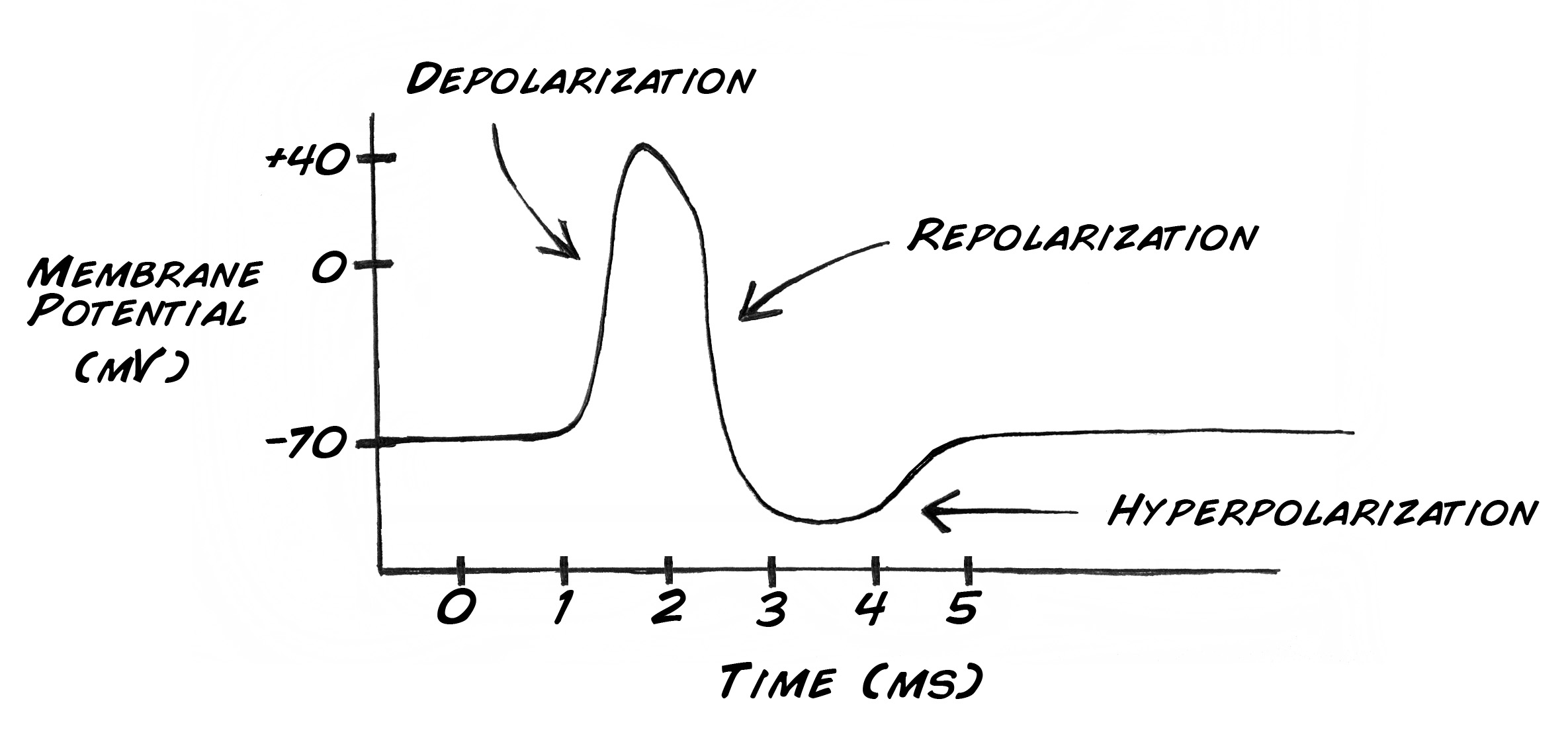
* SpikerBox
* Computer with Audacity installed or iPhone/iPad/Android device with Backyard Brains app
* Associated Laptop Cable or iPhone/Android Cable
* Cockroach
* Dry Ice or access to a freezer
* A candle
* A box approximately 5 – 6 inches taller than the candle
* Digital thermometer
* Dissection scissors
* Toothpick

**INTRODUCTION**

In order to understand the effect that temperature can have on neural communication, one must first understand some specifics about how that communication takes place. We discussed the basics about action potentials (APs) in Lab One; APs are generated at the cell membrane through the movements of various ions (Na+, K+, Cl-) across the membrane. Let’s look closer at the mechanisms of that process to see how temperature factors in.

The cell membrane is a double layer of lipids (i.e. fats) that are hydrophobic (they don’t mix with water). This **lipid bi-layer** is the barrier between the inside of the cell and the outside of the cell, allowing the cell to maintain a different concentration of ions inside the cell than outside the cell. Thus, ions align on both the inner and outer surfaces of the membrane with an excess of positive charges (Na+ ions) on the outside of the membrane and an excess of negative charges (Cl- ions) on the inside. The charge separation creates a difference in electrical potential, or voltage, across the membrane called the **resting membrane potential**. The potential outside the cell is defined as zero, so thus the resting membrane potential is then negative, usually in the range of -60 mV to -70 mV.

How, then, do ions flow through the membrane to produce action potentials? The ion currents are carried into and out of the cells through **ion channels**, proteins that span the cell membrane and can open to conduct specific ions across to the other side. In nerve cells, ion channels conduct ions at extremely high rates (up to 100 million ions per second). Ion channels can be active at rest, or activated due to an external stimulus (such as voltage or chemicals). Resting channels in cell membranes can be selective for Na+, K+, or Cl- ions, and it is in fact the resultant concentration of ions inside and outside of the cell due to these channels that are most responsible for the resting membrane potential. Ion pumps also actively move ions into and out of the cell to maintain that stable membrane potential at rest.

During an action potential, Na+ channels are activated by changes in voltage that allow the Na+ ions layered on the outer cell membrane to rapidly diffuse into the cell and thus push the inner membrane potential highly positive (depolarization). This positive membrane potential causes the Na+ channels to close and K+ channels to instantly open, allowing the K+ ions inside the cell to rapidly diffuse out of the cell and drop the membrane potential sharply in the other direction (repolarization). At this point the K+ channels close, but the K+ outflow current has brought the membrane potential below the resting state (hyperpolarization). With the voltage-gated ion channels closed, the resting channels again dominate and a slower influx of K+ ions returns the cell membrane to its resting state.

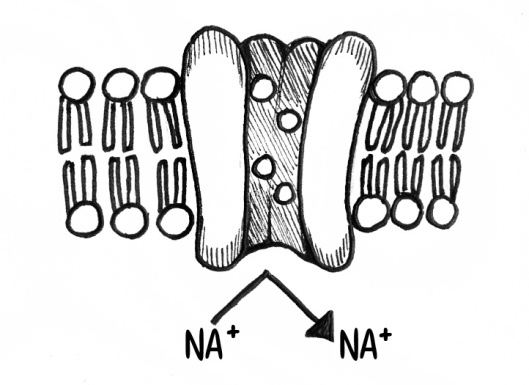
Recent research has demonstrated ion channel activation has a dependence on temperature. Collins et al 1972 demonstrated that the conductance of Na+ channels is dependent upon temperature with an Arrhenius temperature relationship (i.e. an equation to relate the rate constant of chemical reactions to temperature) defined by this equation:

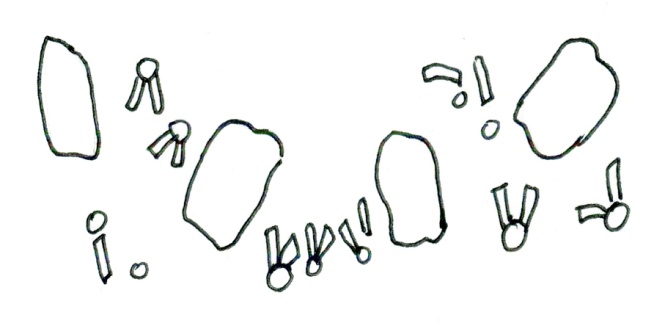
Ln (1/Τ) = ln F – Ea/(RT)

Where T is temperature, Ea is the difference in energy level between an activated ion channel and a non-active state, and both F and R are constants. If we solve that equation for the activation energy,

Ea = RT\*ln(TF)

We see that activation energy increases as T increases, and conversely that it would lower as T decreases. The take-home for our purposes is that the ion channel is more active at higher temperatures (more likely to be open), and less active at lower temperatures. Fohlmeister et al demonstrated in 2009 that sodium channels in retinal ganglion cells were consistently “sleepy” for temperatures below 8 degrees Celsius, with a loss of spiking for T < 7 C (note: different cell types have different temperature ranges, and the neurons in this experiment can spike well below 7 C).

The next question is, why? Why is there such a temperature dependence for neural cell behavior? Wouldn’t it be great if neurons worked just as well at all temperatures? The short answer is that every active cellular process, including the opening and closing of ion channels, is some type of chemical reaction that requires energy. As a general rule of thumb (though there are many exceptions) increasing temperature speeds up and increases chemical reactions, while decreasing the temperature slows them down. At extreme cold temperatures chemical reactions can stop happening altogether, which in this example could lead to ion channels not opening and thus no action potential initiation.

While increasing temperatures can speed up chemical reactions within certain ranges, we also have to consider that if temperature is increased too much this could damage the cell in other ways. The cell membrane is composed of lipids, and lipids transition from solid and ordered (called a gel) to liquid and disordered (called ‘fluid’) based upon temperature. These temperature transition thresholds are different based on the type of lipid and the environment, but once these thresholds are reached the lipid cell membrane can break apart which effectively results in the death of that cell.

Lipid bilayer breaks down at high temperatures

**SETUP**

**Computer Setup**

Use of a computer for the basic aspects of this experiment is not required, as the SpikerBox is equipped with a small speaker. However, in order to perform some of the analysis described in this manual, a computer will be necessary.

There are several free options for capturing and analyzing data from your SpikerBox.

**Backyard Brains IPhone/iPad App:** Backyard Brains iPhone/iPad app, which can be downloaded via iTunes. This app can be found at the following link:

[**Backyard Brains iPhone/iPad app**](http://itunes.apple.com/us/app/backyard-brains/id367151200?mt=8)

To use this app, you will need to purchase or make an audio cable to link the SpikerBox to the iPhone.

**Audacity:** Audacity is a freeware program designed for editing and recording sounds. While not the original purpose of the software, Audacity is a perfect way to convert the amplified signal of the SpikerBox into visual and recordable data. Audacity runs on both Windows, Mac OS X, and Linux/Unix. The website for Audacity can found at the following link:

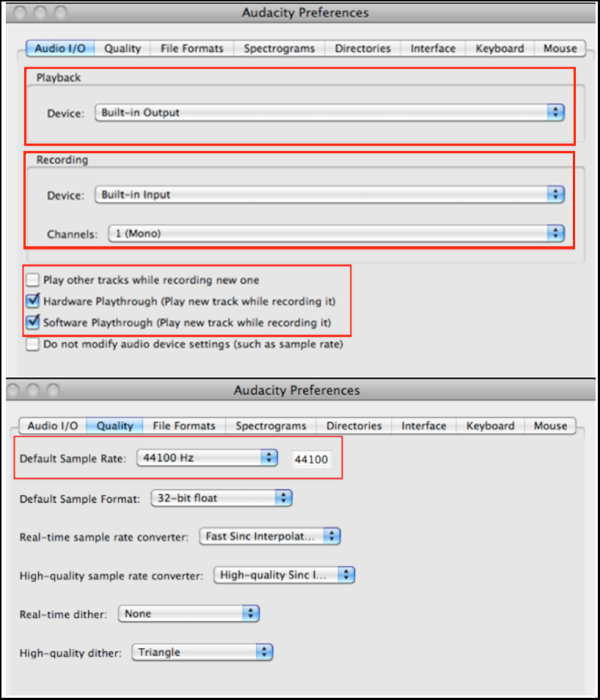
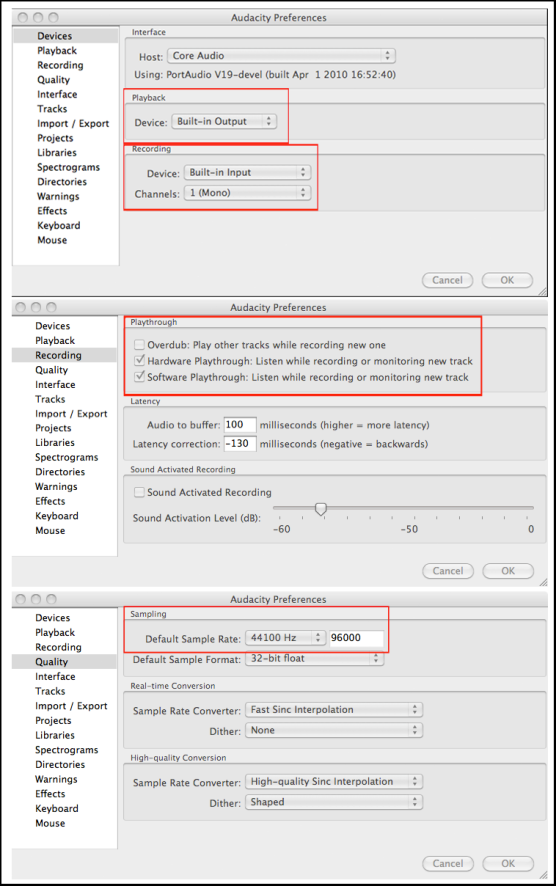
[**Audacity Homepage**](http://audacity.sourceforge.net/)

Once Audacity has been installed, refer to the following procedure:

**Audacity Setup**

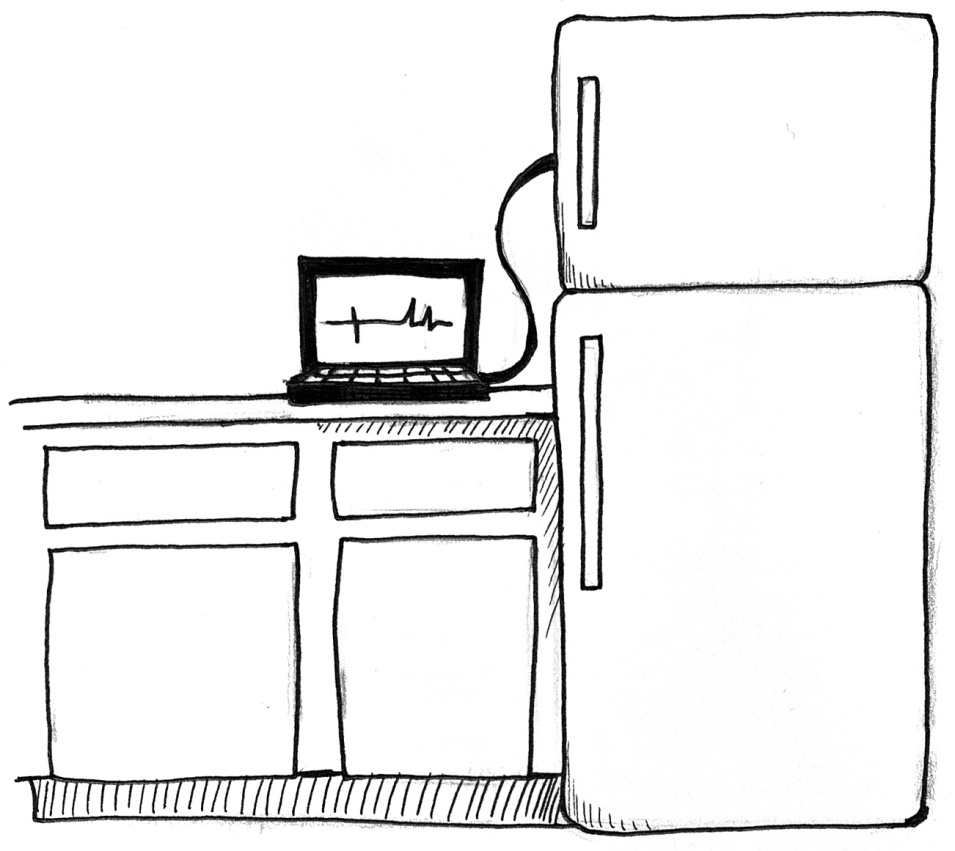
1. If using a laptop, fully charge your battery and record from the SpikerBox while unplugged. When plugged in, theSpikerBox will amplify the current running through the electrical system of the building. The result will be an increase in electrical noise recorded by Audacity. Running off of battery power will decrease the amount of electrical noise.
2. If using a laptop, set the brightness of your screen to maximum. Similar to the affects of the surrounding electrical wiring on your recordings, a dimly lit LCD display will create a significant amount of noise that can be amplified by the SpikerBox.
3. Plug your SpikerBox into the Laptop with male-to-male audio cable. This is exactly the same as a headphone cable, except it has “male” connector on each end.
4. Open Audacity, go to **File 🡪 Preferences**. Note: Due to differences in Audacity between versions, there may be differences in how the **Preferences** window is organized. The primary settings you need to set up are the same, even if they look a little different on your screen.
5. Select the **Audio I/O** tab in the **Preferences** window. Some versions place this window in the **Devices** tab.
6. Select the **Built-in Input** as the **Recording Device** from the drop down menu.
7. Select **1 (Mono)** from the **Channels** drop down menu.
8. Select **Built-in Output** as the **Playback Device** from the drop down menu.
9. The following steps are found either in the **Audio I/O** or **Recording** tabs in the **Preferences** window. The following checkboxes are listed in order as they appear:
   * + **Uncheck** the box: Play other tracks while recording a new one.
     + **Check** the Box: Hardware Playthrough
     + **Check** the box: Software Playthrough
10. Select the tab labeled **Quality**. Set the **Default Sample Rate** to 44100 Hz (or 44,100 times per second).
11. Audacity should now be configured to record from your SpikerBox.

Below are examples of how your **Preferences** window should be configured. Keep in mind that your version of Audacity may look different, but the settings will be the same. Two examples are provided, with the important settings highlighted.

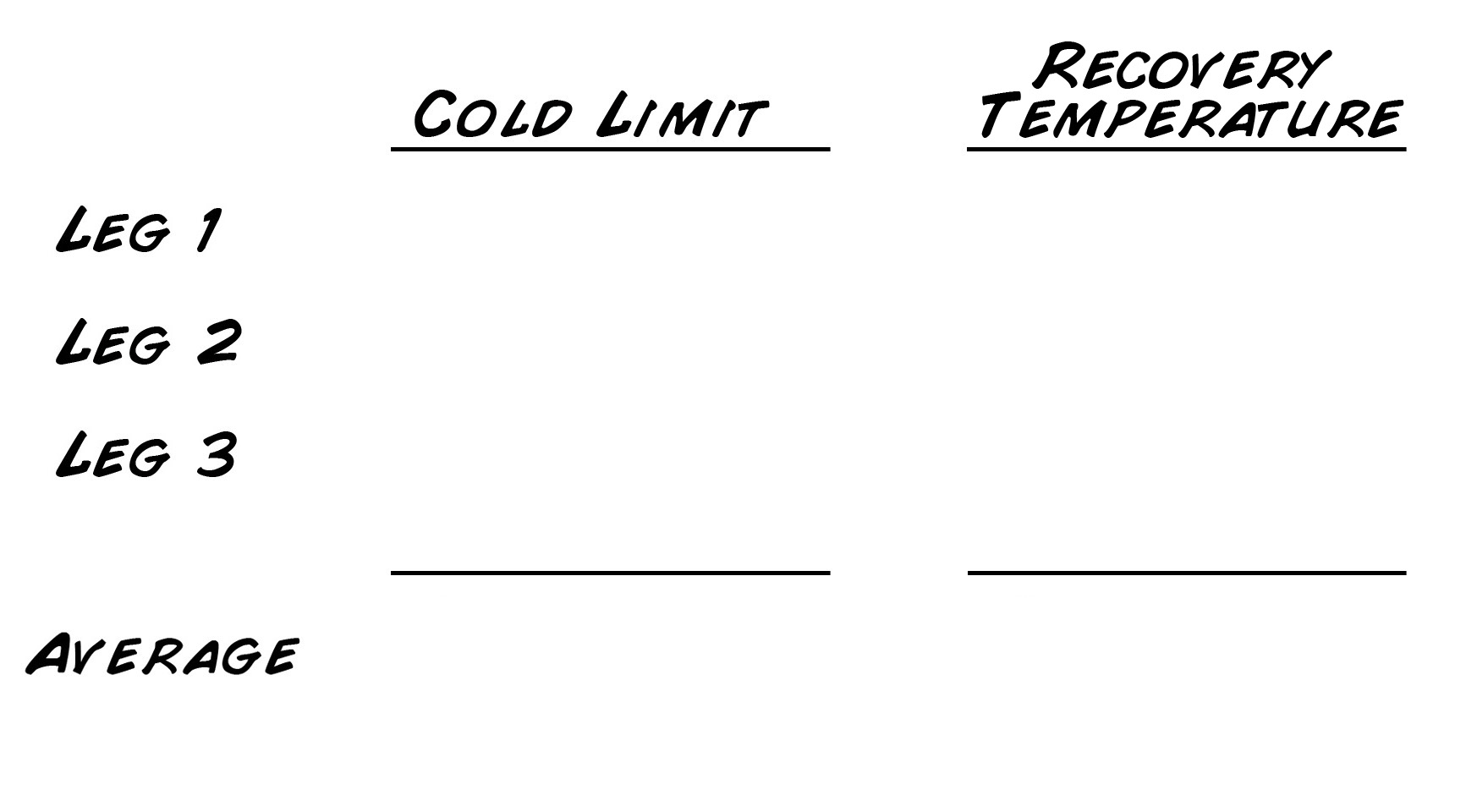


**PROCEDURE**

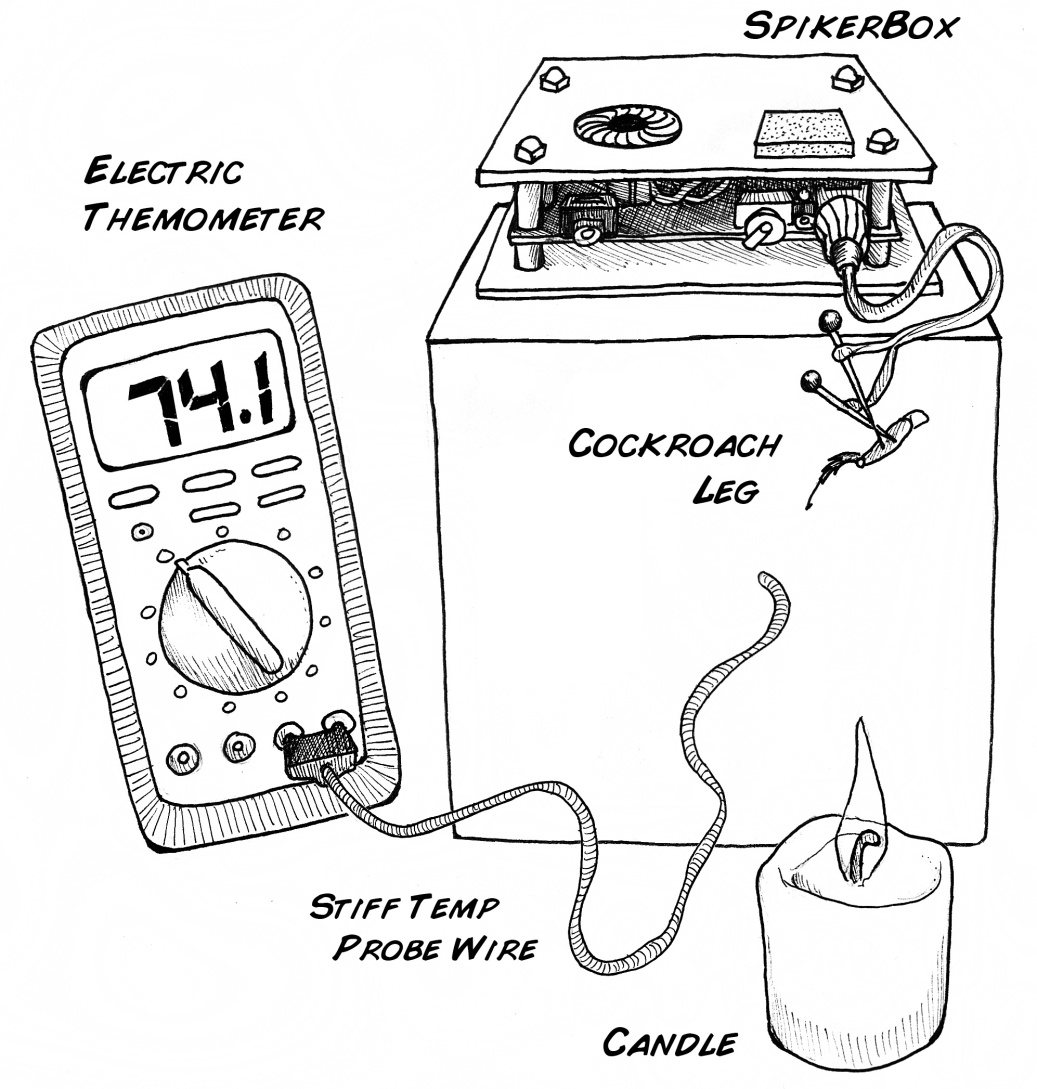
**Exercise 1: Neural Response to Cold**

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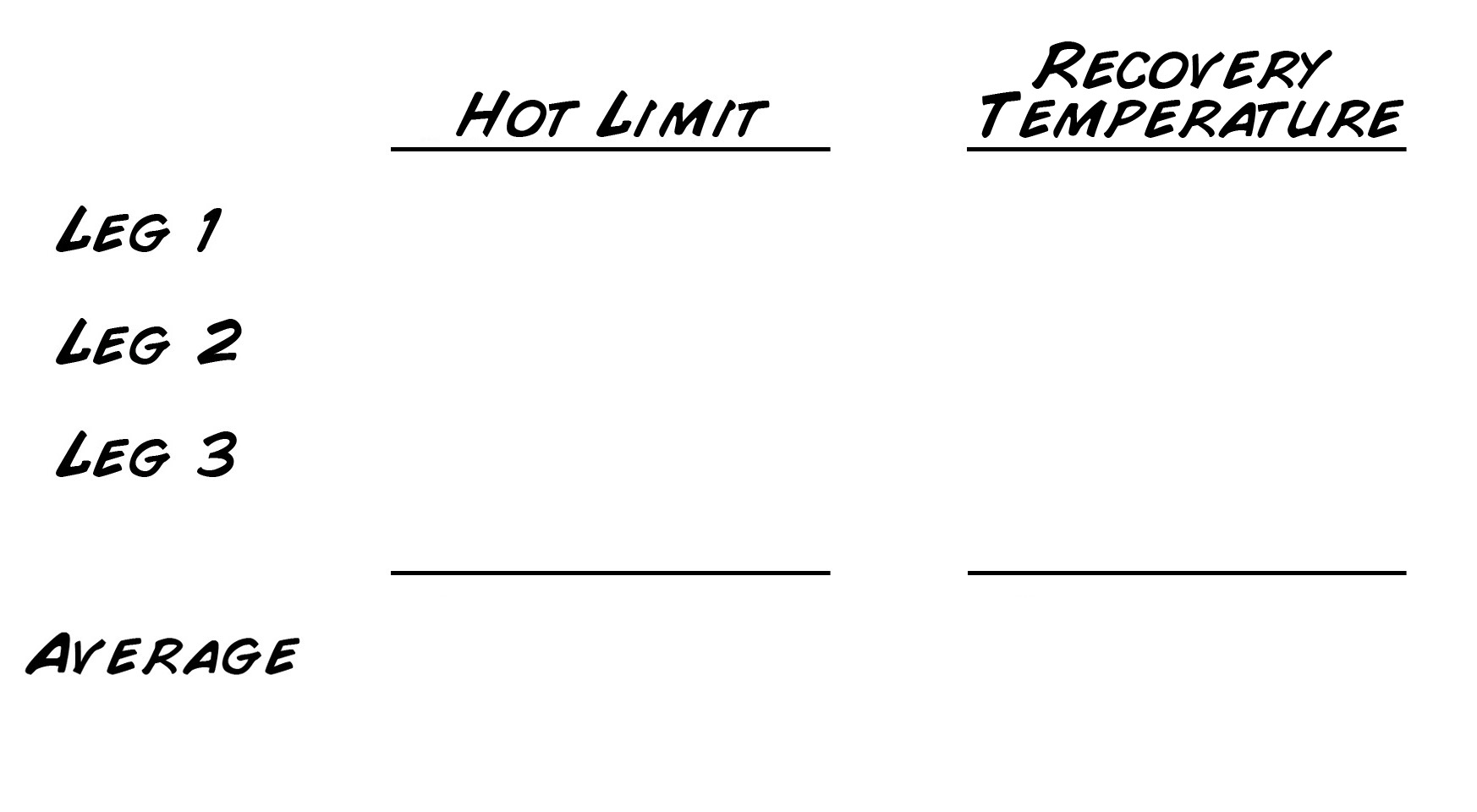
1. Prepare a cockroach leg as described in Lab 1.
2. Place your SpikerBox in the freezer of a household refrigerator. Make sure that the cable is long enough to go from your laptop into the freezer, and stays connected to the SpikerBox you placed inside. Note that actively refrigerators can be very electrically noisy, so you may want to unplug the cold freezer or, as an alternative, use a cooler of dry ice.
3. Place a digital thermometer inside the freezer
4. Listen to the neurons firing for 1 – 3 minutes
5. At a certain temperature the spikes should go away. Open the freezer and record the temperature displayed on the thermometer. Hint, the temperature should be below “freezing”, as typically 31о - 33о F is not cold enough. If you use water ice instead of dry ice in your cooler, your spikes will not go away.
6. Remove your SpikerBox and digital thermometer from the freezer, and listen until the neurons start firing again. Make note of the temperature, and how long it takes before the spikes come back.
7. Repeat steps 1 – 6, three times



**Exercise 2: Neural Response to Heat**



1. Place the SpikerBox on top of the box, and have the electrode with the cockroach leg attached hanging out “in space” 5 – 6 inches over the top of the candle
2. Position the wire of the digital thermometer such that you can measure the precise temperature where the leg is. Record the initial temperature in the room.
3. Light the candle
4. Listen to the neurons firing. As the temperature slowly rises, you should hear the neurons responding
5. At a certain temperature, you will hear the neurons discharge with a sound like a very low scream of spikes. Record the temperature at which this happens, and put out the candle
6. When the thermometer indicates that the leg is back at the initial room temperature, test to see if the spikes come back
7. Repeat steps 1 – 6, three times

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**DISCUSSION QUESTIONS**

1. How did the neurons respond to being placed in low temperatures? Was the response sudden, or gradual? Explain.

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2. On very cold winter days (in some parts of the world) the outside temperature can go below the temperatures used in this experiment, yet animals can still survive and move. What are some possible reasons for why the neurons in these animals don’t stop firing?

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3. How did the neurons respond to being placed in very high temperatures? Was the response sudden, or gradual? Explain.

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4. At both temperature extremes the neuron stops firing, but for one of the extremes the cell resumes firing when returned to room temperature while for the other it doesn’t. Which is which, and what might this difference reflect?

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